# 4' HETEROARYL DIARYLAMINES

This invention relates to 4' heteroaryl diarylamines having pharmaceutical activity.

## BACKGROUND

MEK enzymes are dual specificity kinases involved in, for example, immunomodulation, inflammation, and proliferative diseases such as cancer and restenosis.

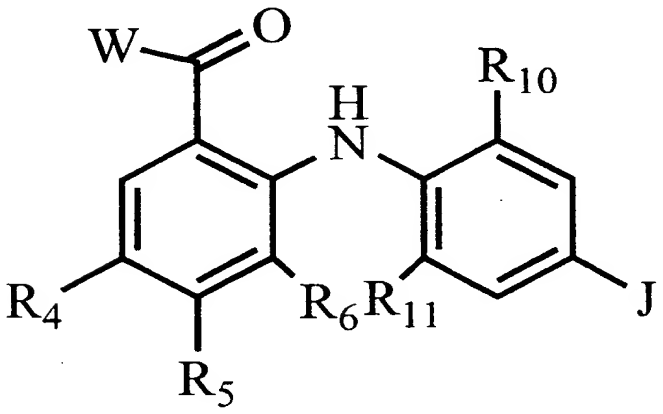
Proliferative diseases are caused by a defect in the intracellular signaling system, or the signal transduction mechanism of certain proteins. Defects include a change either in the intrinsic activity or in the cellular concentration of one or more signaling proteins in the signaling cascade . The cell may produce a growth factor that binds to its own receptors, resulting in an autocrine loop, which continually stimulates proliferation. Mutations or overexpression of intracellular signaling proteins can lead to spurious mitogenic signals within the cell. Some of the most common mutations occur in genes encoding the protein known as Ras, a G-protein that is activated when bound to GTP, and inactivated when bound to GDP. The above-mentioned growth factor receptors, and many other mitogenic receptors, when activated, lead to Ras being converted from the GDP-bound state to the GTP-bound state. This signal is an absolute prerequisite for proliferation in most cell types. Defects in this signaling system, especially in the deactivation of the Ras-GTP complex, are common in cancers, and lead to the signaling cascade below Ras being chronically activated.

Activated Ras leads in turn to the activation of a cascade of serine/threonine kinases. One of the groups of kinases known to require an active Ras-GTP for its own activation is the Raf family. These in turn activate MEK (e.g., $MEK_1$ and $MEK_2$) which then activates MAP kinase, ERK ($ERK_1$ and $ERK_2$). Activation of MAP kinase by mitogens appears to be essential for proliferation; constitutive activation of this kinase is sufficient to induce cellular transformation. Blockade of downstream Ras signaling, for example by use of a

dominant negative Raf-1 protein, can completely inhibit mitogenesis, whether induced from cell surface receptors or from oncogenic Ras mutants. Although Ras is not itself a protein kinase, it participates in the activation of Raf and other kinases, most likely through a phosphorylation mechanism. Once activated, Raf and other kinases phosphorylate MEK on two closely adjacent serine residues, $S^{218}$ and $S^{222}$ in the case of MEK-1, which are the prerequisite for activation of MEK as a kinase. MEK in turn phosphorylates MAP kinase on both a tyrosine, $Y^{185}$, and a threonine residue, $T^{183}$, separated by a single amino acid. This double phosphorylation activates MAP kinase at least 100-fold. Activated MAP kinase can then catalyze the phosphorylation of a large number of proteins, including several transcription factors and other kinases. Many of these MAP kinase phosphorylations are mitogenically activating for the target protein, such as a kinase, a transcription factor, or another cellular protein. In addition to Raf-1 and MEKK, other kinases activate MEK, and MEK itself appears to be a signal integrating kinase. Current understanding is that MEK is highly specific for the phosphorylation of MAP kinase. In fact, no substrate for MEK other than the MAP kinase , ERK, has been demonstrated to date and MEK does not phosphorylate peptides based on the MAP kinase phosphorylation sequence, or even phosphorylate denatured MAP kinase. MEK also appears to associate strongly with MAP kinase prior to phosphorylating it, suggesting that phosphorylation of MAP kinase by MEK may require a prior strong interaction between the two proteins. Both this requirement and the unusual specificity of MEK are suggestive that it may have enough difference in its mechanism of action to other protein kinases that selective inhibitors of MEK, possibly operating through allosteric mechanisms rather than through the usual blockade of the ATP binding site, may be found.

## SUMMARY OF THE INVENTION

The invention features a compound having the formula (I) below:



(I)

In formula (I), W is $OR_1$, $NR_2OR_1$, $NR_AR_B$, $NR_2NR_AR_B$, $O(CH_2)_{1-4}NR_AR_B$, or $NR_2(CH_2)_{1-4}NR_AR_B$. $R_1$ is H, $C_{1-8}$ alkyl, $C_{3-8}$ alkenyl, $C_{3-8}$ alkynyl, $C_{3-8}$ cycloalkyl, phenyl, (phenyl)$C_{1-4}$ alkyl, (phenyl)$C_{3-4}$ alkenyl, (phenyl)-$C_{3-4}$ alkynyl, ($C_{3-8}$ cycloalkyl)$C_{1-4}$ alkyl, ($C_{3-8}$ cycloalkyl)$C_{3-4}$ alkenyl, ($C_{3-8}$ cycloalkyl)$C_{3-4}$ alkynyl, $C_{3-8}$ heterocyclic radical, ($C_{3-8}$ heterocyclic radical)$C_{1-4}$ alkyl, ($C_{3-8}$ heterocyclic radical)$C_{3-4}$ alkenyl, or ($C_{3-8}$ heterocyclic radical)$C_{3-4}$ alkynyl. Each of $R_2$ and $R_3$ is independently H, phenyl, $C_{1-4}$ alkyl, $C_{3-8}$ alkenyl, $C_{3-8}$ alkynyl, $C_{3-8}$ cycloalkyl, or ($C_{3-8}$ cycloalkyl)$C_{1-4}$ alkyl. Each of $R_4$, $R_5$ and $R_6$ is independently H, F, Br, Cl, or $NO_2$. $R_A$ is H, $C_{1-6}$ alkyl, $C_{3-8}$ alkenyl, $C_{3-8}$ alkynyl, $C_{3-8}$ cycloalkyl, phenyl, ($C_{3-8}$ cycloalkyl)$C_{1-4}$ alkyl, ($C_{3-8}$ cycloalkyl)$C_{3-4}$ alkenyl, ($C_{3-8}$ cycloalkyl)$C_{3-4}$ alkynyl, $C_{3-8}$ heterocyclic radical, ($C_{3-8}$ heterocyclic radical)$C_{1-4}$ alkyl, (aminosulfonyl)phenyl, (aminosulfonyl)-phenyl]$C_{1-4}$ alkyl, (aminosulfonyl)$C_{1-6}$ alkyl, (aminosulfonyl)$C_{3-6}$ cycloalkyl, or [(aminosulfonyl)$C_{3-6}$ cycloalkyl]$C_{1-4}$ alkyl. $R_B$ is H, $C_{1-8}$ alkyl, $C_{3-8}$ alkenyl, $C_{3-8}$ alkynyl, $C_{3-8}$ cycloalkyl, or phenyl. J is $SR_C$, $OR_C$, $SO_2R_C$, $SOR_C$, $SO_2NR_DR_E$, C

$_{1-8}$ alkyl, C $_{3-8}$ alkenyl, C $_{3-8}$ alkynyl, C $_{3-8}$ cycloalkyl, C $_{5-8}$ cycloalkenyl, phenyl, (C $_{3-8}$ cycloalkyl)C $_{1-4}$ alkyl, (C $_{3-8}$ cycloalkyl)C $_{3-4}$ alkenyl, (C $_{3-8}$ cycloalkyl)-C $_{3-4}$ alkynyl, C $_{3-8}$ heterocyclic radical (e.g., 1,2,5-thiadiazol-3-yl), (C $_{3-8}$ heterocyclic radical) C $_{1-4}$ alkyl, -M'E'G', (heterocyclic radical)-M'-E'-G', or (cycloalkyl)-M'-E'-G'. M' is O, SO, $SO_2$, $NR_E$, (CO)$NR_E$, $NR_E$ (CO), $SO_2NR_E$, $NR_ESO_2$, or $CH_2$. E' is absent (in other words, a covalent bond), $(CH_2)_{1-4}$ or $(CH_2)_m O(CH_2)_p$ where $1 \leq$ (each of m and p independently) $\leq 3$ and $2 \leq (m + p) \leq 4$. G' is $OR_3$, $SOR_C$, $SO_2R_C$, or $NR_FR_G$; provided that where p = 1, then G' is H. Each of $R_C$, $R_D$, $R_E$, $R_F$ and $R_G$ is independently selected from H, C $_{1-6}$ alkyl, C $_{3-4}$ alkenyl, C $_{3-4}$ alkynyl, C $_{3-6}$ cycloalkyl, C $_{3-6}$ heterocyclic radical, and phenyl; $NR_FR_G$ and $NR_DR_E$ can each also independently be selected from morpholinyl, pyrazinyl, piperazinyl, pyrrolidinyl, or piperadinyl. $R_{10}$ is H, C $_{1-4}$ alkyl, halo, $NO_2$, or $SO_2NR_HR_I$. $R_{11}$ is H, halo, or $NO_2$.

Each hydrocarbon radical or heterocyclic radical above is optionally substituted with between 1 and 3 substituents independently selected from halo, C $_{1-4}$ alkyl, C $_{3-6}$ cycloalkyl, C $_{3-4}$ alkenyl, C $_{3-4}$ alkynyl, phenyl, hydroxy, amino, (amino)sulfonyl, and $NO_2$, wherein each substituent alkyl, cycloalkyl, alkenyl, alkynyl or phenyl is in turn optionally substituted with between 1 and 3 substituents independently selected from halo, C $_{1-2}$ alkyl, hydroxy, amino, and $NO_2$. The invention also encompasses a pharmaceutically acceptable salt or C $_{1-7}$ ester of a compound of formula (I).

The invention also relates to a pharmaceutical composition including (a) a compound of formula (I) and (b) a pharmaceutically-acceptable carrier.

The invention further relates to a method for treating proliferative diseases, such as cancer, restenosis, psoriasis, autoimmune disease, and atherosclerosis. Other aspects of the invention include methods for treating MEK-related (including ras-related) cancers, whether solid or hematopoietic. Examples of cancers include colorectal, cervical, breast, ovarian, brain, acute leukemia, gastric, non-small cell lung, pancreatic, prostatic, and renal cancers. Further aspects of the invention include methods for treating or reducing the symptoms of xenograft (cell(s), llimb, skin, organ or bone marrow transplant) rejection, osteoarthritis, rheumatoid arthritis, cystic fibrosis, complications of diabetes

(including diabetic retinopathy and diabetic nephropathy), hepatomegaly, cardiomegaly, stroke (such as acute focal ischemic stroke and global cerebral ischemia), heart failure, septic shock, asthma, and Alzheimer's disease. Compounds of the invention are also useful as antiviral agents for treating viral infections such as HIV, hepatitis (B) virus (HBV), human papilloma virus (HPV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). These methods include the step of administering to a patient in need of such treatment, or suffering from such a disease or condition, a pharmaceutically-effective amount of a disclosed compound or pharmaceutical composition thereof.

The invention also features methods of combination therapy, such as a method for treating cancer, wherein the method further includes providing radiation therapy or chemotherapy, for example, with mitotic inhibitors such as a taxane or a vinca alkaloid. Examples of mitotic inhibitors include paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, and vinflunine Other therapeutic combinations include a MEK inhibitor of the invention and an anticancer agent such as cisplatin, 5-fluoro-2-4(1H,3H)-pyrimidinedione (5FU), flutamide, and gemcitabine.

The chemotherapy or radiation therapy may be administered before, concurrently, or after the administration of a disclosed compound according to the needs of the patient.

The invention further includes synthetic intermediates and methods disclosed herein.

Other aspects of the invention are provided in the description, examples, and claims below.

## DETAILED DESCRIPTION

The invention features 4-heteroaryl diarylamine compounds, pharmaceutical compositions thereof, and methods of using such compounds and compositions.

According to one aspect of the invention, the compounds are MEK inhibitors. MEK inhibition assays include the in vitro MEK/MAP described at column 6, line 36 to column 7, line 4 of U.S. Patent Number 5,525,625 and the in

vitro MEK assay described at column 7, lines 4-27 of the same patent, the entire disclosure of which is incorporated by reference (see also Examples 13 et seq.). A whole cell assay is described in Example 16.

**A. Terms**

Certain terms are defined below and by their usage throughout this disclosure.

Alkyl groups include aliphatic (i.e., hydrocarbyl or hydrocarbon radical structures containing hydrogen and carbon atoms) with a free valence. Alkyl groups are understood to include straight chain and branched structures. Examples include methyl, ethyl, propyl, isopropyl, butyl, n-butyl, isobutyl, t-butyl, pentyl, isopentyl, 2,3-dimethylpropyl, hexyl, 2,3-dimethylhexyl, 1,1-dimethylpentyl, heptyl, and octyl. Cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl.

Alkyl groups can be substituted with 1, 2, 3 or more substituents which are independently selected from halo (fluoro, chloro, bromo, or iodo), hydroxy, amino, alkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aryloxy, arylalkyloxy, heterocyclic radical, and (heterocyclic radical)oxy. Specific examples include fluoromethyl, hydroxyethyl, 2,3-dihydroxyethyl, (2- or 3-furanyl)methyl, cyclopropylmethyl, benzyloxyethyl, (3-pyridinyl)methyl, (2- or 3-furanyl)methyl, (2-thienyl)ethyl, hydroxypropyl, aminocyclohexyl, 2-dimethylaminobutyl, methoxymethyl, *N*-pyridinylethyl, diethylaminoethyl, and cyclobutylmethyl.

Alkenyl groups are analogous to alkyl groups, but have at least one double bond (two adjacent $sp^2$ carbon atoms). Depending on the placement of a double bond and substituents, if any, the geometry of the double bond may be *entgegen* (E), or *zusammen* (Z), *cis*, or *trans*. Similarly, alkynyl groups have at least one triple bond (two adjacent sp carbon atoms). Unsaturated alkenyl or alkynyl groups may have one or more double or triple bonds, respectively, or a mixture thereof; like alkyl groups, unsaturated groups may be straight chain or branched, and they may be substituted as described both above for alkyl groups and throughout the disclosure by example. Examples of alkenyls, alkynyls, and substituted forms include cis-2-butenyl, trans-2-butenyl, 3-butynyl, 3-phenyl-2-

propynyl, 3-(2'-fluorophenyl)-2-propynyl, 3-methyl(5-phenyl)-4-pentynyl, 2-hydroxy-2-propynyl, 2-methyl-2-propynyl, 2-propenyl, 4-hydroxy-3-butynyl, 3-(3-fluorophenyl)-2-propynyl, and 2-methyl-2-propenyl. In formula (I), alkenyls and alkynyls can be $C_{2-4}$ or $C_{2-8}$, for example, and are preferably $C_{3-4}$ or $C_{3-8}$.

More general forms of substituted hydrocarbon radicals include hydroxyalkyl, hydroxyalkenyl, hydroxyalkynyl, hydroxycycloalkyl, hydroxyaryl, and corresponding forms for the prefixes amino-, halo- (e.g., fluoro-, chloro-, or bromo-), nitro-, alkyl-, phenyl-, cycloalkyl- and so on, or combinations of substituents. According to formula (I), therefore, substituted alkyls include hydroxyalkyl, aminoalkyl, nitroalkyl, haloalkyl, alkylalkyl (branched alkyls, such as methylpentyl), (cycloalkyl)alkyl, phenylalkyl, alkoxy, alkylaminoalkyl, dialkylaminoalkyl, arylalkyl, aryloxyalkyl, arylalkyloxyalkyl, (heterocyclic radical)alkyl, and (heterocyclic radical)oxyalkyl. $R_1$ thus includes hydroxyalkyl, hydroxyalkenyl, hydroxyalkynyl, hydroxycycloalkyl, hydroxyaryl, aminoalkyl, aminoalkenyl, aminoalkynyl, aminocycloalkyl, aminoaryl, alkylalkenyl, (alkylaryl)alkyl, (haloaryl)alkyl, (hydroxyaryl)alkynyl, and so forth. Similarly, $R_A$ includes hydroxyalkyl and aminoaryl, and $R_B$ includes hydroxyalkyl, aminoalkyl, and hydroxyalkyl(heterocyclic radical)alkyl.

Heterocyclic radicals, which include but are not limited to heteroaryls, include: furyl, oxazolyl, isoxazolyl, thiophenyl, thiazolyl, pyrrolyl, imidazolyl, 1,3,4-triazolyl, tetrazolyl, pyridinyl, pyrimidinyl, pyridazinyl, indolyl, 1,2,5-thiadiazolyl and their nonaromatic counterparts. Further examples of heterocyclic radicals include piperidyl, quinolyl, isothiazolyl, piperidinyl, morpholinyl, piperazinyl, tetrahydrofuryl, tetrahydropyrrolyl, pyrrolidinyl, octahydroindolyl, thiadiazolyl, octahydrobenzothiofuranyl, and octahydrobenzofuranyl.

Selective MEK 1 or MEK 2 inhibitors are those compounds which inhibit the MEK 1 or MEK 2 enzymes, respectively, without substantially inhibiting other enzymes such as MKK3, PKC, Cdk2A, phosphorylase kinase, EGF, and PDGF receptor kinases, and C-src. In general, a selective MEK 1 or MEK 2 inhibitor has an $IC_{50}$ for MEK 1 or MEK 2 that is at least one-fiftieth (1/50) that of its $IC_{50}$ for one of the above-named other enzymes. Preferably, a selective inhibitor has an $IC_{50}$ that is at least 1/100, more preferably 1/500, and even more preferably

1/1000, 1/5000, or less than that of its $IC_{50}$ or one or more of the above-named enzymes.

**B. Compounds**

One aspect of the invention features disclosed compounds shown in formula (I) in the Summary section. Embodiments of the invention include compounds wherein: (a) $R_C$ is C $_{1-2}$ alkyl; (b) W is OH, or W is $NHOR_1$ (c) $R_{10}$ is methyl or chloro; (d) $R_{11}$ is fluoro; (e) $R_{11}$ is H; (f) J is trihalomethyl or methylthio; (g) J is $SO_2CH_3$; (h) J is $SOCH_3$; (i) J is C $_{3-8}$ alkynyl where the triple bond is between the carbon atoms alpha and beta to the phenyl group; (j) $R_1$ has at least one hydroxy substituent; (k) $R_1$ is H, methyl, ethyl, propyl, isopropyl, isobutyl, benzyl, phenethyl, allyl, C $_{3-5}$ alkenyl, C $_{3-5}$ alkynyl, C $_{3-6}$ cycloalkyl, (C $_{3-5}$ cycloalkyl)C $_{1-2}$ alkyl, or (C $_{3-5}$ heterocyclic radical)C $_{1-2}$ alkyl; (l) $R_1$ is H or (C $_{3-4}$ cycloalkyl)C $_{1-2}$ alkyl; (m) $R_2$ is H, methyl, C $_{3-4}$ alkynyl, C $_{3-5}$ cycloalkyl, or (C $_{3-5}$ cycloalkyl)methyl; (n) $R_A$ is H, methyl, ethyl, isobutyl, hydroxyethyl, hydroxypropyl, cyclopropylmethyl, cyclobutylmethyl, C $_{3-4}$ alkynyl, phenyl, 2-piperidin-1-yl-ethyl, 2,3-dihydroxy-propyl, 3-[4-(2-hydroxyethyl)-piperazin-1-yl]-propyl, 2-pyrrolidin-1-yl-ethyl, or 2-diethylamino-ethyl; and $R_B$ is H; or where $R_B$ is methyl and $R_A$ is phenyl; (o) each of $R_4$ and $R_6$ is H, and $R_5$ is F; (p) each of $R_4$, $R_5$, and $R_6$ is F; (q) $R_5$ is F; (r) each $R_5$ and $R_6$ is F and $R_6$ is Br; (s) each $R_5$ and $R_6$ is F and $R_6$ is H; (t) J is 1,2,5-thiadiazol-3-yl; or a combination thereof.

Preferably, where one of $R_1$, $R_2$, $R_A$, $R_B$, $R_C$, $R_D$, $R_E$, $R_F$, and $R_G$, for example, is an alkenyl or alkynyl group, its double or triple bond, respectively, is not adjacent the point of attachment. For example, where W is $NR_2OR_1$, $R_2$ is preferably prop-2-ynyl, or but-2 or 3-enyl, and less preferably prop-1-ynyl or but-1-enyl.

Examples of compounds of formula (I) include: 4-fluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 5-bromo-3,4-difluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 3,4-difluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 2-(4-methanesulfinyl-2-methyl-phenylamino)-4-nitro-benzoic acid; 3,4,5-trifluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-

benzoic acid; 3,4-difluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 2-(2-methyl-4-methylsulfanyl-phenylamino)-4-nitro-benzoic acid; 3,4,5-trifluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 4-fluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 5-bromo-3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzoic acid; 3,4,5-trifluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 4-fluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 5-bromo-3,4-difluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzoic acid; and 2-(4-methanesulfonyl-2-methyl-phenylamino)-4-nitro-benzoic acid; and the corresponding hydroxamic acid or cyclopropylhydroxamic acid of each.

Preferred examples of compounds of formula (I) are : 4-Fluoro-2-(4-methanesulfanyl-phenylamino)-benzoic acid (**1**); 4-Fluoro-2-(4-methanesulfinyl-phenylamino)-benzoic acid (**2**); 4-Fluoro-2-(4-methanesulfonyl-phenylamino)-benzoic acid (**3**); 4-Fluoro-2-(2-methyl-4-trimethylsilanylethynyl-phenylamino)-benzoic acid **(6);** 4-Fluoro-2-(2-methyl-4-ethynyl-phenylamino)-benzoic acid (**7**). Biological data on these seven compounds is given on page 17; full characterization of the compounds - MP, NMR, MS, IR and CHN- is given on pages 28-31.

Additional preferred compounds include the following: (a) 5-Bromo-2-(4-ethynyl-2-methyl-phenylamino)-3,4-difluoro-benzoic acid; N-Cyclopropylmethoxy-2-(4-ethynyl-2-methyl-phenylamino)-3,4-difluoro-benzamide; 2-(4-Ethynyl-2-methyl-phenylamino)-3,4-difluoro-benzoic acid; N-Cyclopropylmethoxy-2-(4-ethynyl-2-methyl-phenylamino)-3,4,5-trifluoro-benzamide; 2-(4-Ethynyl-2-methyl-phenylamino)-3,4,5-trifluoro-benzoic acid; 5-Bromo-N-cyclopropylmethoxy-2-(4-ethynyl-2-methyl-phenylamino)-3,4-difluoro-benzamide; (b) 5-Bromo-2-(4-ethynyl-Cl-methyl-phenylamino)-3,4-difluoro-benzoic acid; N-Cyclopropylmethoxy-2-(4-ethynyl-Cl-methyl-phenylamino)-3,4-difluoro-benzamide; 2-(4-Ethynyl-Cl-methyl-phenylamino)-3,4-difluoro-benzoic acid; N-Cyclopropylmethoxy-2-(4-ethynyl-Cl-methyl-phenylamino)-3,4,5-trifluoro-benzamide; 2-(4-Ethynyl-Cl-methyl-phenylamino)-3,4,5-trifluoro-benzoic acid; 5-Bromo-N-cyclopropylmethoxy-2-(4-ethynyl-Cl-methyl-phenylamino)-3,4-difluoro-benzamide; (c) 5-bromo-3,4-difluoro-

2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 3,4-difluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzoic acid; 3,4,5-trifluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzoic acid; 3,4-difluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzoic acid; 5-bromo-3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzoic acid; 3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzoic acid; (d) 5-bromo-N-cyclopropylmethoxy-3,4-difluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4-difluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4,5-trifluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4-difluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4,5-trifluoro-2-(4-methanesulfinyl-2-methyl-phenylamino)-benzamide; 5-bromo-N-cyclopropylmethoxy-3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4,5-trifluoro-2-(2-methyl-4-methylsulfanyl-phenylamino)-benzamide; N-cyclopropylmethoxy-3,4-difluoro-2-(4-methanesulfonyl-2-methyl-phenylamino)-benzamide; (e) N-cyclopropylmethoxy-3,4-difluoro-2-(4-imidazol-1-yl-2-methyl-phenylamino)-benzamide; (f) N-cyclopropylmethoxy-3,4,5-trifluoro-2-(2-methyl-4-[1,2,5]thiadiazol-3-yl-phenylamino)-benzamide; 2-[4-(4-chloro-[1,2,5]thiadiazol-3-yl)-2-methyl-phenylamino]-3,4,5-trifluoro-benzoic acid; 2-[4-(4-chloro-[1,2,5]thiadiazol-3-yl)-2-methyl-phenylamino]-N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; (g) 2-{4-[4-(2-dimethylamino-ethoxy)-[1,2,5]thiadiazol-3-yl]-2-methyl-phenylamino}-3,4,5-trifluoro-benzoic acid; (h) N-cyclopropylmethoxy-3,4,5-trifluoro-2-{2-methyl-4-[4-(2-piperidin-1-yl-ethoxy)-[1,2,5]thiadiazol-3-yl]-phenylamino}-benzamide.

Further preferred compounds include: (a) 5-bromo-2-(2-chloro-4-methylsulfanyl-phenylamino)-3,4-difluoro-benzoic acid; 2-(2-chloro-4-methanesulfinyl-phenylamino)-3,4-difluoro-benzoic acid; 2-(2-chloro-4-methanesulfonyl-phenylamino)-3,4,5-trifluoro-benzoic acid; 2-(2-chloro-methylsulfanyl-phenylamino)-3,4-difluoro-benzoic acid; 5-bromo-2-(2-chloro-4-methanesulfonyl-phenylamino)-3,4-difluoro-benzoic acid; 2-(2-Chloro-4-methanesulfonyl-phenylamino)-3,4-difluoro-benzoic acid; (b) 5-bromo-2-(2-

chloro-4-methylsulfanyl-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-chloro-4-methanesulfinyl-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-chloro-4-methanesulfonyl-phenylamino)- N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 2-(2-chloro-4-methylsulfanyl-phenylamino)- N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-chloro-4-methanesulfinyl-phenylamino)- N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 5-bromo-2-(2-chloro-4-methanesulfonyl-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-chloro-4-methylsulfanyl-phenylamino)-N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 2-(2-chloro-4-methanesulfonyl-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide; and (c) 2-[2-chloro 4-(3H-imidazol-1-yl)-phenylamino]-N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-chloro-4-[1,2,5]thiadiazol-3-yl-phenylamino)-N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 2-[4-(2-chloro-4-chloro-[1,2,5]thiadiazol-3-yl)-phenylamino]-3,4,5-trifluoro-benzoic acid; 2-[2-chloro-4-(4-chloro-[1,2,5]thiadiazol-3-yl)-phenylamino]-N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 2-{4-[4-(2-dimethylamino-ethoxy)-[1,2,5]thiadiazol-3-yl]-2-methyl-phenylamino}-3,4,5-trifluoro-benzoic acid; 2-{2-chloro-4-[4-(2-piperidin-1-yl-ethoxy)-[1,2,5]thiadiazol-3-yl]-phenylamino}-N-cyclopropylmethoxy-3,4,5-trifluoro-benzamide;

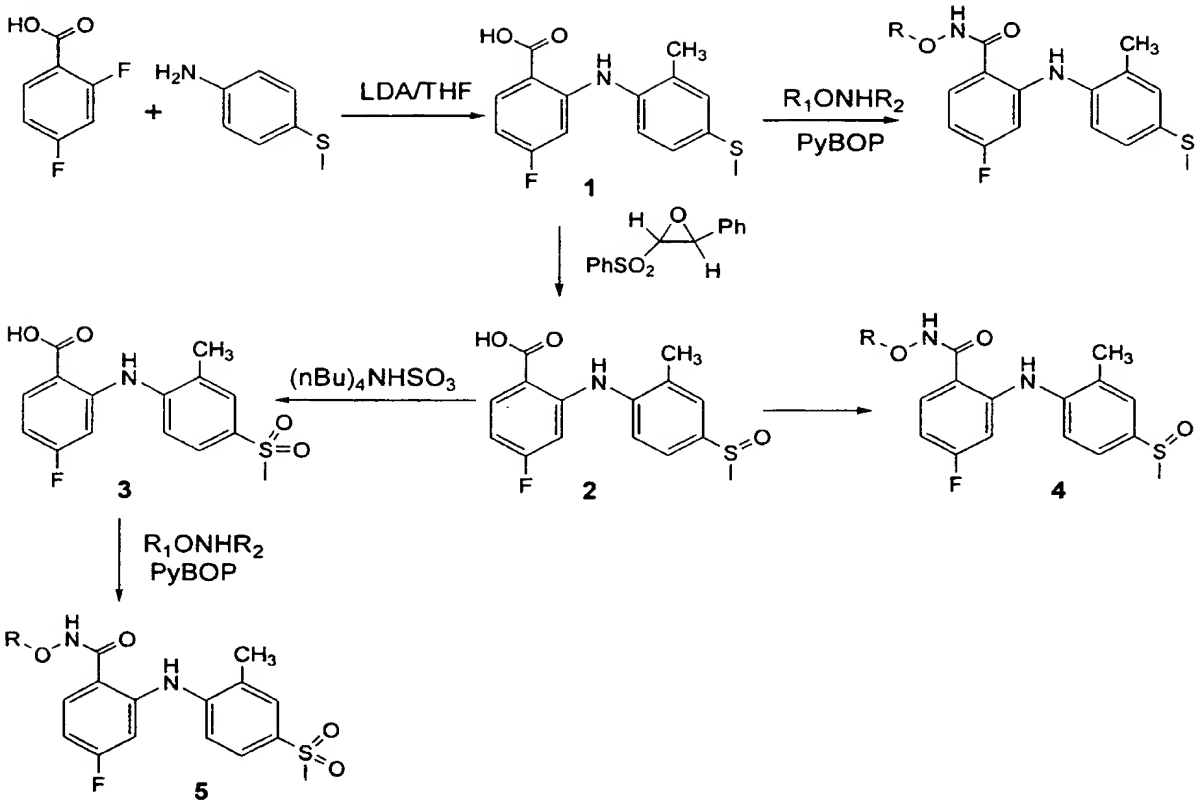
Additional preferred compounds include: (a) 2-(2-Chloro-4-ethynyl-phenylamino)-4-fluoro-benzoic acid; 5-Bromo-2-(2-chloro-4-ethynyl-phenylamino)-3,4-difluoro-benzoic acid; 2-(2-Chloro-4-ethynyl-phenylamino)- N-cyclopropylmethoxy-3,4-difluoro-benzamide; 2-(2-Chloro-4-ethynyl-phenylamino)-N-cyclopropylmethoxy-4-nitro-benzamide; 2-(2-Chloro-4-ethynyl-phenylamino)-N-hydroxy-3,4,5-trifluoro- benzamide; 2-(2-Chloro-4-ethynyl-phenylamino)-3,4-difluoro-benzoic acid; 2-(4-Ethynyl-2-chloro-phenylamino)-4-nitro-benzoic acid; 2-(2-Chloro-4-ethynyl-phenylamino)- N-Cyclopropylmethoxy-3,4,5-trifluoro-benzamide; 2-(2-chloro-4-methanesulfinyl-phenylamino)- 4-fluoro-N-hydroxy-benzamide; 5-Bromo-2-(4-ethynyl-2-chloro-phenylamino)-3,4-difluoro-N-hydroxy-benzamide; (b) 2-(2-Chloro-4-ethynyl-phenylamino)-3,4,5-trifluoro-benzoic acid; 2-(2-Chloro-4-ethynyl-phenylamino)- N-cyclopropylmethoxy-4-fluoro-benzamide; 5-Bromo-2-(2-chloro-4-ethynyl-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-

benzamide; 2-(4-Ethynyl-2-chloro-phenylamino)-3,4-difluoro-N-hydroxy-benzamide; 2-(4-Ethynyl-2-chloro-phenylamino)-N-hydroxy-4-nitro-benzamide; and (c) 2-(2-Chloro-4-ethynyl-phenylamino)-4-fluoro-benzoic acid; 2-(2-Chloro-4-ethynyl-phenylamino)- N-cyclopropylmethoxy-4-fluoro-benzamide; 2-(2-Chloro-4-methanesulfinyl-phenylamino)- 4-fluoro-N-hydroxy-benzamide; 2-(2-chloro-4-imidazol-1-yl-phenylamino)- 3,4-Difluoro-benzoic acid.

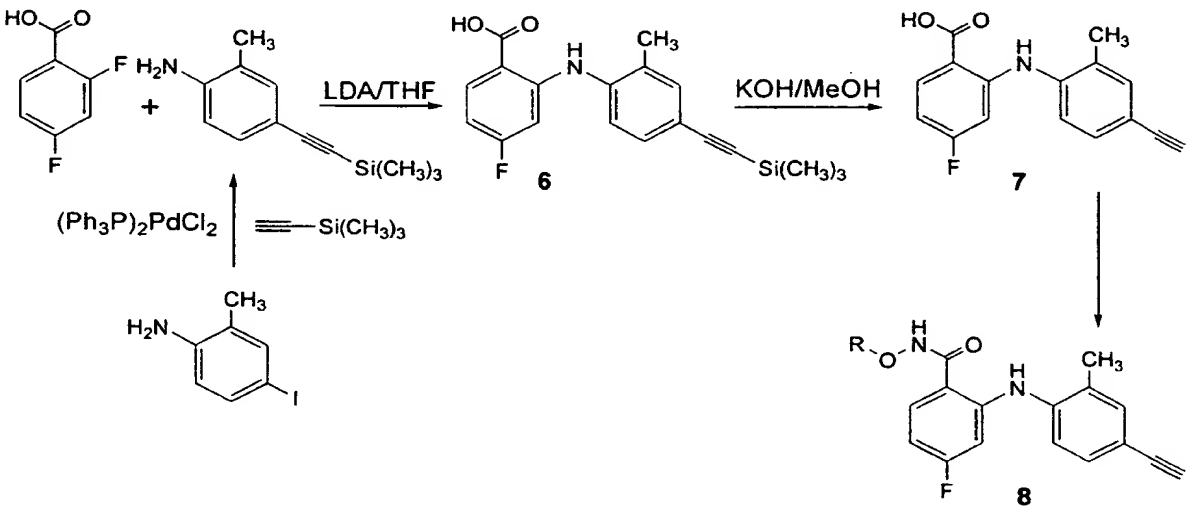
**C. Synth sis**

The disclosed compounds can be synthesized according to the following five schemes, or variants thereof. The abbreviation PyBOP is (benzotriazolyl-oxy)-tripyrrolidino phosphonium hexafluorophosphate. These synthetic strategies are further exemplified in Examples 1-12 below:

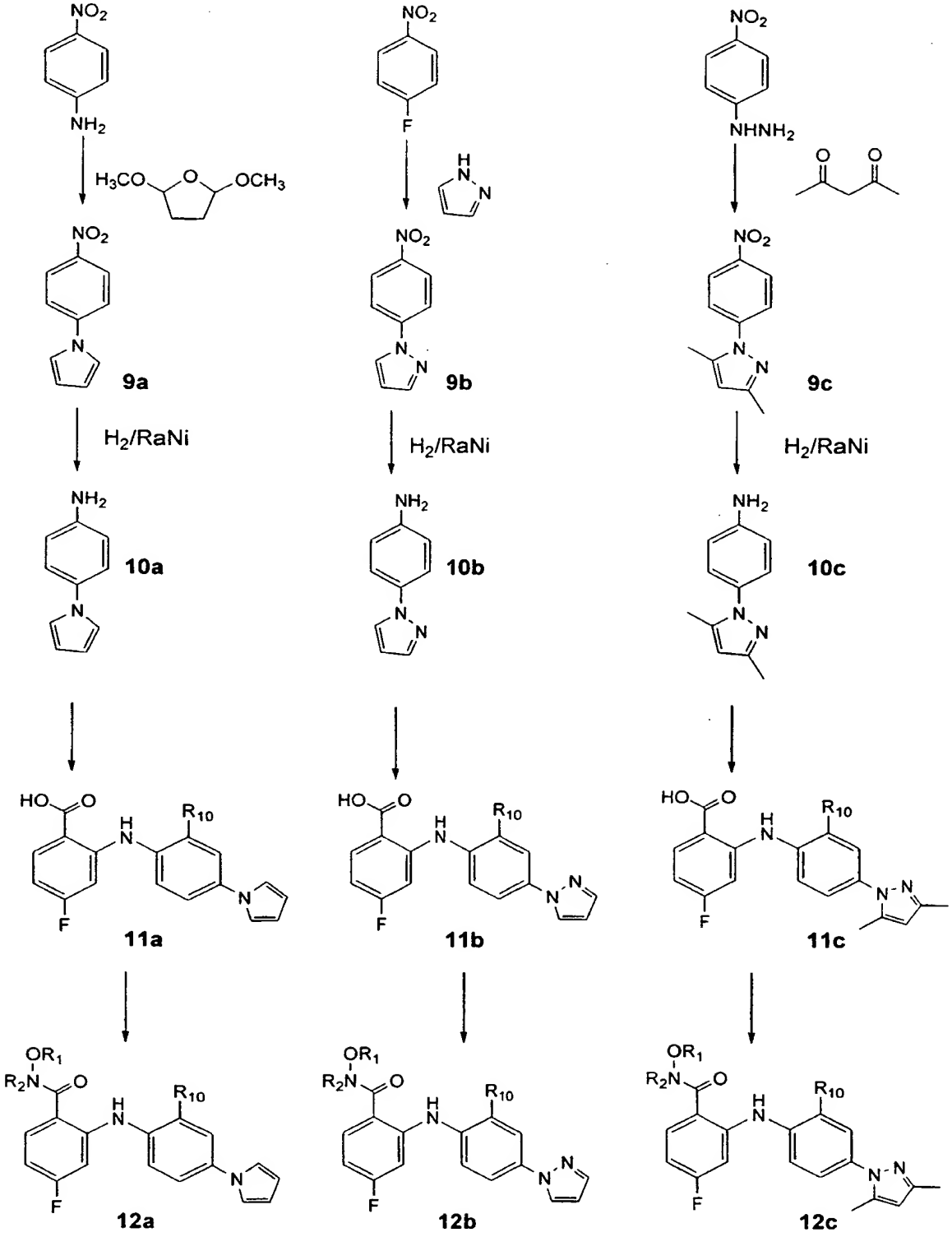
**Schem 1**



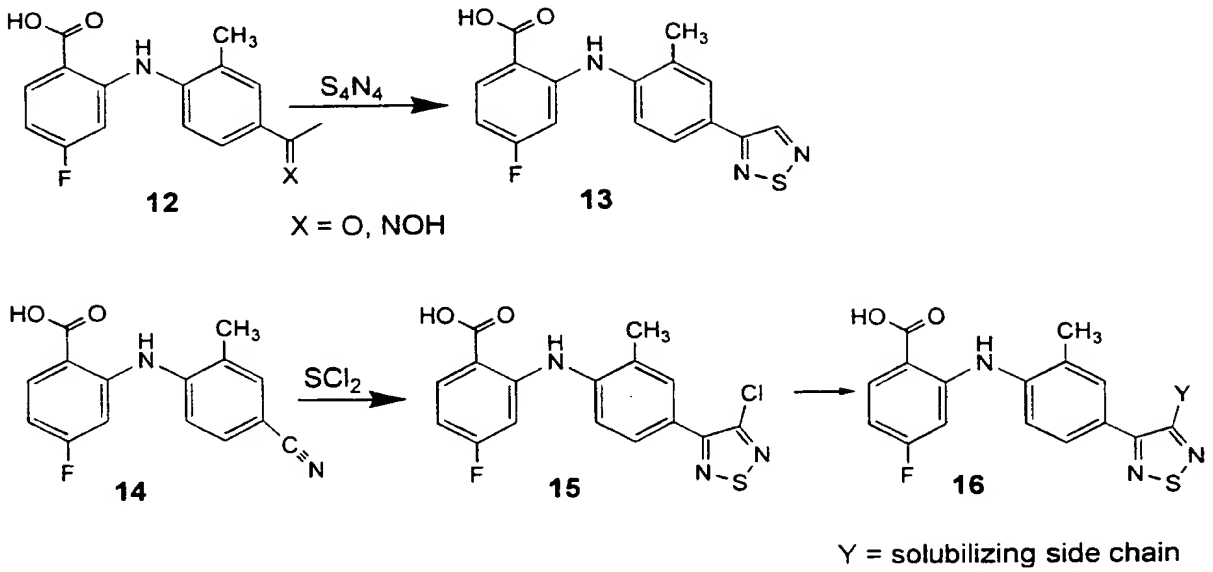

**Scheme 2**



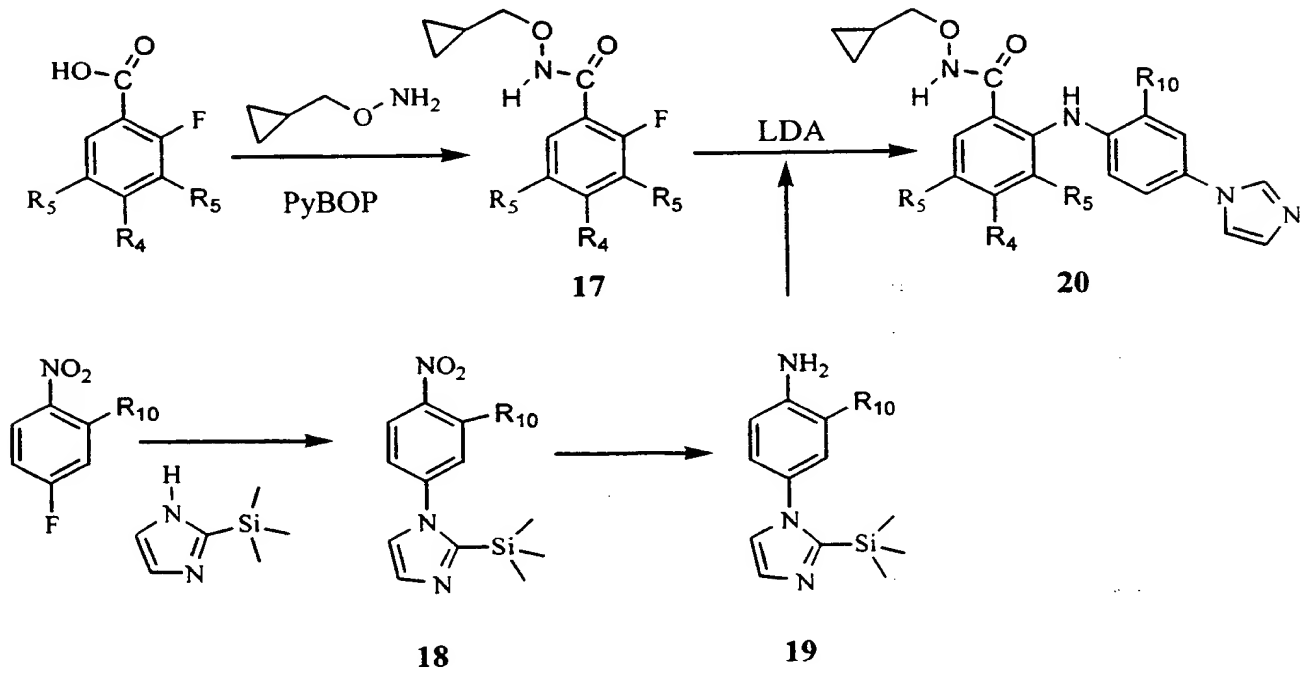
**Scheme 3**


NO2
NH2
H3CO
O
OCH3
F
NHNH2
9a
9b
9c
H2/RaNi
10a
10b
10c
HO
R10
11a
11b
11c
OR1
R2N
12a
12b
12c

**Scheme 4** 3-Aryl-1,2,5-thiadiazols




**Scheme 5**



**D. Us s**

The disclosed compositions are useful as both prophylactic and therapeutic treatments for diseases or conditions as provided in the Summary section, as well as diseases or conditions modulated by the MEK cascade. Examples include stroke, heart failure, osteoarthritis, rheumatoid arthritis, organ transplant rejection, and a variety of tumors such as ovarian, non-small cell lung, pancreatic, brain, prostatic, renal, and colon.

MEK INHIBITION

| COMPOUND | $IC_{50}$ |
|---|---|
| 4-Fluoro-2-(4-methanesulfanyl-phenylamino)-benzoic acid (**1**) | 1.93 μM |
| 4-Fluoro-2-(4-methanesulfinyl-phenylamino)-benzoic acid (**2**) | > 1 μM |
| 4-Fluoro-2-(4-methanesulfonyl-phenylamino)-benzoic acid (**3**) | 316 nM |
| 4-Fluoro-2-(2-methyl-4-trimethylsilanylethynyl-phenylamino)-benzoic acid **(6)** | > 10 μM |
| 4-Fluoro-2-(2-methyl-4-ethynyl-phenylamino)-benzoic acid (**7**) | 272 nM |

1. Dosages

Those skilled in the art will be able to determine, according to known methods, the appropriate dosage for a patient, taking into account factors such as age, weight, general health, the type of symptoms requiring treatment, and the presence of other medications. In general, an effective amount will be between 0.1 and 1000 mg/kg per day, preferably between 1 and 300 mg/kg body weight, and daily dosages will be between 10 and 5000 mg for an adult subject of normal weight. Capsules, tablets or other formulations (such as liquids and film-coated tablets) may be of between 5 and 200 mg, such as 10, 15, 25, 35, 50 mg, 60 mg, and 100 mg and can be administered according to the disclosed methods.

2. Formulations

Dosage unit forms include tablets, capsules, pills, powders, granules, aqueous and nonaqueous oral solutions and suspensions, and parenteral solutions packaged in containers adapted for subdivision into individual doses. Dosage unit forms can also be adapted for various methods of administration, including controlled release formulations, such as subcutaneous implants. Administration methods include oral, rectal, parenteral (intravenous, intramuscular, subcutaneous), intracisternal, intravaginal, intraperitoneal, intravesical, local (drops, powders, ointments, gels, or cream), and by inhalation (a buccal or nasal spray).

Parenteral formulations include pharmaceutically acceptable aqueous or nonaqueous solutions, dispersion, suspensions, emulsions, and sterile powders for the preparation thereof. Examples of carriers include water, ethanol, polyols (propylene glycol, polyethylene glycol), vegetable oils, and injectable organic esters such as ethyl oleate. Fluidity can be maintained by the use of a coating such as lecithin, a surfactant, or maintaining appropriate particle size. Carriers for solid dosage forms include (a) fillers or extenders, (b) binders, (c) humectants, (d) disintegrating agents, (e) solution retarders, (f) absorption acccelerators, (g) adsorbants, (h) lubricants, (i) buffering agents, and (j) propellants.

Compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents; antimicrobial agents such as parabens, chlorobutanol, phenol, and sorbic acid; isotonic agents such as a sugar or sodium chloride; absorption-prolonging agents such as aluminum monostearate and gelatin; and absorption-enhancing agents.

3. Related compounds

The invention provides the disclosed compounds and closely related, pharmaceutically acceptable forms of the disclosed compounds, such as salts, esters, amides, hydrates or solvated forms thereof; masked or protected forms; and racemic mixtures, or enantiomerically or optically pure forms.

Pharmaceutically acceptable salts, esters, and amides include carboxylate salts (e.g., $C_{1-8}$ alkyl, cycloalkyl, aryl, heteroaryl, or non-aromatic heterocyclic),

amino acid addition salts, esters, and amides which are within a reasonable benefit/risk ratio, pharmacologically effective, and suitable for contact with the tissues of patients without undue toxicity, irritation, or allergic response. Representative salts include hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactiobionate, and laurylsulfonate. These may include alkali metal and alkali earth cations such as sodium, potassium, calcium, and magnesium, as well as non-toxic ammonium, quaternary ammonium, and amine cations such as tetramethyl ammonium, methylamine, trimethylamine, and ethylamine. See, for example, S.M. Berge, et al., "Pharmaceutical Salts," *J. Pharm. Sci.,* 1977, 66:1-19 which is incorporated herein by reference. Representative pharmaceutically acceptable amides of the invention include those derived from ammonia, primary $C_{1-6}$ alkyl amines and secondary di ($C_{1-6}$ alkyl) amines. Secondary amines include 5- or 6-membered heterocyclic or heteroaromatic ring moieties containing at least one nitrogen atom and optionally between 1 and 2 additional heteroatoms. Preferred amides are derived from ammonia, $C_{1-3}$ alkyl primary amines, and di ($C_{1-2}$ alkyl)amines. Representative pharmaceutically acceptable esters of the invention include $C_{1-7}$ alkyl, $C_{5-7}$ cycloalkyl, phenyl, and phenyl($C_{1-6}$)alkyl esters. Preferred esters include methyl esters.

The invention also includes disclosed compounds having one or more functional groups (e.g., hydroxyl, amino, or carboxyl) masked by a protecting group. Some of these masked or protected compounds are pharmaceutically acceptable; others will be useful as intermediates. Synthetic intermediates and processes disclosed herein, and minor modifications thereof, are also within the scope of the invention.

HYDROXYL PROTECTING GROUPS

Hydroxyl protecting groups include: ethers, esters, and protection for 1,2- and 1,3-diols. The ether protecting groups include: methyl, substituted methyl

ethers, substituted ethyl ethers, substituted benzyl ethers, silyl ethers and conversion of silyl ethers to other functional groups.

Substituted Methyl Ethers

Substituted methyl ethers include: methoxymethyl, methylthiomethyl, *t*- butylthiomethyl, (phenyldimethylsilyl) methoxymethyl, benzyloxymethyl, *p*-ethoxybenzyloxymethyl, (4-methoxyphenoxy) methyl, guaiacolmethyl, *t*-butoxymethyl, 4-pentenyloxymethyl, siloxymethyl, 2-methoxyethoxymethyl, 2,2,2-trichloroethoxymethyl, bis(2-chloro- ethoxy)methyl, 2-(trimethylsilyl)-ethoxymethyl, tetrahydropyranyl, 3-bromotetrahydro-pyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl, 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl *S,S*-dioxido, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl, 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, and 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-ethanobenzofuran-2-yl.

Substituted Ethyl Ethers

Substituted ethyl ethers include: 1-ethoxyethyl, 1-(2,chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilyethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, and benzyl.

Substituted Benzyl Ethers

Substituted benzyl ethers include: *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2- and 4-picolyl, 3-methyl-2-picolyl *N*-oxido, diphenylmethyl, *p*, *p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyl-diphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)-phenylmethyl, tri-(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxy)phenyl-diphenylmethyl, 4,4',4"-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4"-tris(levulinoyloxyphenyl) methyl, 4,4',4"tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-ylmethyl)bis(4',4"-dimethoxyphenyl)-methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl) xanthenyl, 9-(9-phenyl-10-oxo) anthryl, 1,3-benzodithiolan-2-yl, and benzisothiazolyl *S,S*-dioxido.

Silyl Ethers

Silyl ethers include: trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylthexylsilyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl, tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, and *t*-butylmethoxyphenylsilyl.

ESTERS

Esters protecting groups include: esters, carbonates, assisted cleavage, miscellaneous esters, and sulfonates.

Esters

Examples of protective esters include: formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, *p*-P-phenylacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio) pentanoate, pivaloate, adamantoate,crotonate,4-methoxycrotonate, benzoate, *p*-phenylbenzoate, and 2,4,6-trimethylbenzoate (mesitoate).

Carbonates

Carbonates include: methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl) ethyl, 2-(phenylsulfonyl) ethyl, 2-(triphenylphosphonio) ethyl, isobutyl, vinyl, allyl, *p*-nitrophenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl, and methyl dithiocarbonate.

Assisted Cleavage

Examples of assisted cleavage protecting groups include: 2-iodobenzoate, 4-azido-butyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl) benzoate, 2-formylbenzene-sulfonate, 2-(methylthiomethoxy) ethyl carbonate, 4-(methylthiomethoxymethyl) benzoate, and 2-(methylthiomethoxymethyl) benzoate.

Miscellaneous Esters

In addition to the above classes, miscellaneous esters include: 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl) phenoxyacetate,

2,4-bis(1,1-dimethylpropyl) phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate (tigloate), *o*-(methoxycarbonyl) benzoate, *p*-P-benzoate, α-naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, *N*-phenylcarbamate, borate, dimethylphosphinothioyl, and 2,4-dinitrophenylsulfenate.

Sulfonates

Protective sulfates includes: sulfate, methanesulfonate(mesylate), benzylsulfonate, and tosylate.

PROTECTION FOR 1,2- AND 1,3-DIOLS

The protection for 1,2 and 1,3-diols group includes: cyclic acetals and ketals, cyclic ortho esters, and silyl derivatives.

Cyclic Acetals and Ketals

Cyclic acetals and ketals include: methylene, ethylidene, 1-*t*-butylethylidene, 1-phenylethylidene, (4-methoxyphenyl) ethylidene, 2,2,2-trichloroethylidene, acetonide (isopropylidene), cyclopentylidene, cyclohexylidene, cycloheptylidene, benzylidene, *p*-methoxybenzylidene, 2,4-dimethoxybenzylidene, 3,4-dimethoxybenzylidene, and 2-nitrobenzylidene.

Cyclic Ortho Esters

Cyclic ortho esters include: methoxymethylene, ethoxymethylene, dimethoxy-methylene, 1-methoxyethylidene, 1-ethoxyethylidine, 1,2-dimethoxyethylidene, α-methoxybenzylidene, 1-(*N,N*-dimethylamino)ethylidene derivative, α-(*N,N*-dimethylamino) benzylidene derivative, and 2-oxacyclopentylidene.

PROTECTION FOR THE CARBOXYL GROUP

ESTERS

Ester protecting groups include: esters, substituted methyl esters, 2-substituted ethyl esters, substituted benzyl esters, silyl esters, activated esters, miscellaneous derivatives, and stannyl esters.

Substituted Methyl Esters

Substituted methyl esters include: 9-fluorenylmethyl, methoxymethyl, methylthiomethyl, tetrahydropyranyl, tetrahydrofuranyl, methoxyethoxymethyl, 2-(trimethylsilyl)ethoxy-methyl, benzyloxymethyl, phenacyl, *p*-bromophenacyl, α-methylphenacyl, *p*-methoxyphenacyl, carboxamidomethyl, and *N*-phthalimidomethyl.

2-Substituted Ethyl Esters

2-Substituted ethyl esters include: 2,2,2-trichloroethyl, 2-haloethyl, |-chloroalkyl, 2-(trimethylsily)ethyl, 2-methylthioethyl, 1,3-dithianyl-2-methyl, 2(*p*-nitrophenylsulfenyl)-ethyl, 2-(*p*-toluenesulfonyl)ethyl, 2-(2′-pyridyl)ethyl, 2-(diphenylphosphino)ethyl, 1-methyl-1-phenylethyl, *t*-butyl, cyclopentyl, cyclohexyl, allyl, 3-buten-1-yl, 4-(trimethylsily)-2-buten-1-yl, cinnamyl, α-methylcinnamyl, phenyl, *p*-(methylmercapto)-phenyl, and benzyl.

Substituted Benzyl Esters

Substituted benzyl esters include: triphenylmethyl, diphenylmethyl, bis(*o*-nitrophenyl)methyl, 9-anthrylmethyl, 2-(9,10-dioxo)anthrylmethyl, 5-dibenzo-suberyl, 1-pyrenylmethyl,2-(trifluoromethyl)-6-chromylmethyl, 2,4,6-trimethylbenzyl, *p*-bromobenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-methoxybenzyl, 2,6-dimethoxybenzyl, 4-(methylsulfinyl)benzyl, 4-sulfobenzyl, piperonyl, and 4-P-benzyl.

Silyl Esters

Silyl esters include: trimethylsilyl, triethylsilyl, *t*-butyldimethylsilyl, *i*-propyldimethylsilyl, phenyldimethylsilyl, and di- *t*-butylmethylsilyl.

Miscellaneous Derivatives

Miscellaneous derivatives includes: oxazoles, 2-alkyl-1,3-oxazolines, 4-alkyl-5-oxo-1,3-oxazolidines, 5-alkyl-4-oxo-1,3-dioxolanes, ortho esters, phenyl group, and pentaaminocobalt(III) complex.

Stannyl Esters

Examples of stannyl esters include: triethylstannyl and tri-*n*-butylstannyl.

AMIDES AND HYDRAZIDES

Amides include: *N,N* –dimethyl, pyrrolidinyl, piperidinyl, 5,6-dihydrophenanthridinyl, *o*-nitroanilides, *N*-7-nitroindolyl, *N*-8-nitro-1,2,3,4-tetrahydroquinolyl, and *p*-P-benzenesulfonamides. Hydrazides include: *N*-phenyl, *N,N'*-diisopropyl and other dialkyl hydrazides.

PROTECTION FOR THE AMINO GROUP

CARBAMATES

Carbamates include: carbamates, substituted ethyl, assisted cleavage, photolytic cleavage, urea-type derivatives, and miscellaneous carbamates.

Carbamates

Carbamates include: methyl and ethyl, 9-fluorenylmethyl, 9-(2-sulfo)fluorenylmethyl, 9-(2,7-dibromo)fluorenylmethyl, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydro- thioxanthyl)]methyl, and 4-methoxyphenacyl.

Substituted Ethyl

Substituted ethyl protective groups include: 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-phenylethyl, 1-(1-adamantyl)-1-methylethyl, 1,1-dimethyl-2-haloethyl, 1,1dimethyl-2,2-dibromoethyl, 1,1-dimethyl-2,2,2-trichloroethyl, 1-methyl-1-(4-biphenylyl)ethyl, 1-(3,5-di-*t*-butylphenyl)-1-methylethyl, 2-(2'-and 4'-pyridyl)ethyl, 2-(*N,N*-icyclohexylcarboxamido)- ethyl, *t*-butyl, 1-adamantyl, vinyl, allyl, 1-isopropylallyl, connamyl, 4-nitrocinnamyl, quinolyl, *N*-hydroxypiperidinyl, alkyldithio, benzyl, *p*-methoxybenzyl, *p*-nitrobenzyl, *p*-bromobenzyl, *p*-chlorobenzyl, 2,4-dichlorobenzyl, 4-methylsulfinylbenzyl, 9-anthrylmethyl, and diphenylmethyl.

Assisted Cleavage

Protection via assisted cleavage includes: 2-methylthioethyl, 2-methylsulfonylethyl, 2-(*p*-toluenesulfonyl)ethyl, [2-(1,3-dithianyl)]methyl, 4-methylthiophenyl, 2,4-dimethyl-thiophenyl, 2-phosphonioethyl, 2-triphenylphosphonioisopropyl, 1,1-dimethyl-2-cyanoethyl, *m*-chloro-*p*-acyloxybenzyl, *p*-(dihydroxyboryl)benzyl, 5-benzisoxazolyl-methyl, and 2-(trifluoromethyl)-6-chromonylmethyl.

Photolytic Cleavage

Photolytic cleavage methods use groups such as: *m*-nitrophenyl, 3,5-dimethoxybenzyl, *o*-nitrobenzyl, 3,4-dimethoxy-6-nitrobenzyl, and phenyl(*o*-nitrophenyl)methyl.

Urea-Type Derivatives

Examples of urea-type derivatives include: phenothiazinyl-(10)-carbonyl derivative, *N'*-p-toluenesulfonylaminocarbonyl, and *N'*-phenylaminothiocarbonyl.

Miscellaneous Carbamates

In addition to the above, miscellaneous carbamates include: *t*-amyl, *S*-benzyl thiocarbamate, *p*-cyanobenzyl, cyclobutyl, cyclohexyl, cyclopentyl, cyclopropylmethyl, *p*-decyloxy-benzyl, diisopropylmethyl, 2,2-dimethoxycarbonylvinyl, *o*-(*N,N*-dimethyl-carboxamido)-benzyl, 1,1-dimethyl-3(*N,N*-dimethylcarboxamido)propyl, 1,1-dimethyl-propynyl, di(2-pyridyl)methyl, 2-furanylmethyl, 2-iodoethyl, isobornyl, isobutyl, isonicotinyl, *p*(*p'*-methoxyphenylazo)benzyl, 1-methylcyclobutyl, 1-methylcyclohexyl, 1-methyl-1-cyclopropylmethyl, 1-methyl-(3,5-dimethoxyphenyl)ethyl, 1-methyl-1(*p*-henylazophenyl)- ethyl, 1-methyl-1-phenylethyl, 1-methyl-1-(4-pyridyl)ethyl, phenyl, *p*-(phenylazo)benzyl, 2,4,6-tri-*t*-butylphenyl, 4-trimethylammonium)-benzyl, and 2,4,6-trimethylbenzyl.

AMIDES

Amides

Amides includes: *N*-formyl, *N*-acetyl, *N*-chloroacetyl, *N*-trichloroacetyl, *N*-trifluoroacetyl, *N*-phenylacetyl, *N*-3-phenylpropionyl, *N*-picolinoyl, *N*-3-pyridylcarboxamide, *N*-benzoylphenylalanyl derivative, *N*-benzoyl, and *N*-*p*-phenylbenzoyl.

Assisted Cleavage

Assisted cleavage groups include: *N*-*o*-nitrophenylacetyl, *N*-*o*-nitrophenoxyacetyl, *N*-acetoacetyl, (*N'*-dithiobenzyloxycarbonylamino)acetyl, *N*-3-

(*p*-hydroxphenyl) propionyl, *N*-3-(*o*-nitrophenyl)propionyl, *N*-2-methyl-2-(*o*-nitrophenoxy)propionyl, *N*-2-methyl-2-(*o*-phenylazophenoxy)propionyl, *N*-4-chlorobutyryl, *N*-3-methyl-3-nitrobutyryl, *N*-*o*-nitrocinnamoyl, *N*-acetylmethionine derivative, *N*-*o*-nitrobenzoyl, *N*-*o*-(benzoyloxymethyl)benzoyl, and 4,5-diphenyl-3-oxazolin-2-one.

Cyclic Imide Derivatives

Cyclic imide derivatives include: *N*-phthalimide, *N*-dithiasuccinoyl, *N*-2,3-diphenyl-maleoyl, *N*-2,5-dimethylpyrrolyl, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct, 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, and 1-substituted 3,5-dinitro-4-pyridonyl.

SPECIAL -NH PROTECTIVE GROUPS

Protective groups for – NH include: *N*-alkyl and *N*-aryl amines, imine derivatives, enamine derivatives, and *N*-hetero atom derivatives (such as *N*-metal, N-N, N-P, N-Si, and N-S), *N*-sulfenyl, and *N*-sulfonyl.

*N*-Alkyl and *N*-Aryl Amines

*N*-alkyl and *N*-aryl amines include: *N*-methyl, *N*-allyl, *N*-[2-(trimethylsilyl)ethoxyl]-methyl, *N*-3-acetoxypropyl, *N*-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl), quaternary ammonium salts, *N*-benzyl, *N*-di(4-methoxyphenyl)methyl, *N*-5-dibenzosuberyl, *N*-triphenylmethyl, *N*-(4-methoxyphenyl)diphenylmethyl, *N*-9-phenylfluorenyl, *N*-2,7-dichloro-9-fluorenylmethylene, *N*-ferrocenylmethyl, and *N*-2-picolylamine *N* '-oxide.

Imine Derivatives

Imine derivatives include: *N*-1,1-dimethylthiomethylene, *N*-benzylidene, *N*-*p*-methoxybenzylidene, *N*-diphenylmethylene, *N*-[(2-pyridyl)mesityl]methylene, *N*-(*N* ',*N* '-dimethylaminomethylene), *N*,*N* '-isopropylidene, *N*-*p*-nitrobenzylidene, *N*-salicylidene, *N*-5-chlorosalicylidene, *N*-(5-chloro-2-hydroxyphenyl)phenyl-methylene, and *N*-cyclohexylidene.

Enamine Derivative

An example of an enamine derivative is *N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl).

*N*-Hetero Atom Derivatives

*N*-metal derivatives include: *N*-borane derivatives, *N*-diphenylborinic acid derivative, *N*-[phenyl(pentacarbonylchromium- or -tungsten)]carbenyl, and *N*-copper or *N*-zinc chelate. Examples of *N*-*N* derivatives include: *N*-nitro, *N*-nitroso, and *N*-oxide. Examples of *N*-P derivatives include: *N*-diphenylphosphinyl, *N*-dimethylthiophosphinyl, *N*-diphenylthiophosphinyl, *N*-dialkyl phosphoryl, *N*-dibenzyl phosphoryl, and *N*-diphenyl phosphoryl. Examples of *N*-sulfenyl derivatives include: *N*-benzenesulfenyl, *N*-*o*-nitrobenzenesulfenyl, *N*-2,4-dinitrobenzenesulfenyl, *N*-pentachlorobenzenesulfenyl, *N*-2-nitro-4-methoxy-benzenesulfenyl, *N*-triphenylmethylsulfenyl, and *N*-3-nitropyridinesulfenyl.

*N*-sulfonyl derivatives include: *N*-*p*-toluenesulfonyl, *N*-benzenesulfonyl, *N*-2,3,6-trimethyl- 4-methoxybenzenesulfonyl, *N*-2,4,6-trimethoxybenzenesulfonyl, *N*-2,6-dimethyl-4-methoxy-benzenesulfonyl, *N*-pentamethylbenzenesulfonyl, *N*-2,3,5,6-tetramethyl-4-methoxybenzene- sulfonyl, *N*-4-methoxybenzenesulfonyl, *N*-2,4,6-trimethylbenzenesulfonyl, *N*-2,6-dimethoxy- 4-methylbenzenesulfonyl, *N*-2,2,5,7,8-pentamethylchroman-6-sulfonyl, *N*-methanesulfonyl, *N*-$\beta$-trimethylsilylethanesulfonyl, *N*-9-anthracenesulfonyl, *N*-4-(4',8'-dimethoxynaphthylmethyl)-benzenesulfonyl, *N*-benzylsulfonyl, *N*-trifluoromethylsulfonyl, and *N*-phenacylsulfonyl.

Disclosed compounds which are masked or protected may be prodrugs, compounds metabolized or otherwise transformed *in vivo* to yield a disclosed compound, e.g., transiently during metabolism. This transformation may be a hydrolysis or oxidation which results from contact with a bodily fluid such as blood, or the action of acids, or liver, gastrointestinal, or other enzymes.

Features of the invention are further described in the examples below.

## E. EXAMPLES

### EXAMPLE 1

4-Fluoro-2-(4-methanesulfanyl-phenylamino)-benzoic acid (**1**).

To a solution of 4-(methylmercapto)aniline (3.1622 g, 0.02 mole) in THF at ¯78°C, a solution of LDA in THF (2M, 30 ml, 0.06 mole) was added and the reaction mixture stirred for 30 minutes at ¯78°C (Scheme 1) . Solid 2,4-diflluoro benzoic acid (3.1622 g, 0.02 mole) was added and the reaction stirred for 16 hours while it wormed up to room temperature. The reaction mixture was pour in to ether saturated with HCl gas. HCl gas was bubbled into until precipitation of salts ceased. The precipitated salts were separated by filtration and discarded. The ether layer was concentrated to give **1** as a white solid. Yield 5.63 g (100%); mp 173-179 °C (DEC); $^{1}$H-NMR (400 MHz; $CDCl_3$) ™ 9.39 (s, 1H), 8.04 (dd, 1H, J=9.2, 6.8 Hz), 7.32-7.17 (AB quartet, 4H), 6.74 (dd, 1H, J=12.1, 2.4 Hz), 6.46-6.41 (m, 1H), 2.51 (s, 3H); $^{13}$C-NMR (100 MHz; $CDCl_3$) ™ 172.79, 167.57 (d, $J_{C-F}$=253.4 Hz), 151.55 (d, $J_{C-F}$=12.2 Hz), 136.83, 135.40 (d, $J_{C-F}$=12.2 Hz), 134.72, 128.31, 124.60, 106.51, 105.12 (d, $J_{C-F}$=22.9 Hz), 99.79 (d, $J_{C-F}$=26.7 Hz), 16.51; $^{19}$F-NMR (376 MHz; $CDCl_3$) ™ -101.39 to -101.46 (m); MS (APCI+) 278 (M+1, 100); IR (KBr) 3319, 1664, 1589, 1258 $cm^{-1}$; Anal. calcd/found for: $C_{14}H_{12}FNO_2S$ C, 60.64/60.99; H, 4.36/4.63; N, 5.05/4.80; S, 11.56/10.97.

### EXAMPLE 2

4-Fluoro-2-(4-methanesulfinyl-phenylamino)-benzoic acid (**2**).

A mixture of **1** (Scheme 1) (0.286 g, 0.001031 mole) and oxaziridine (0.235 g, 0.0009 mole) in $CHCl_3$ (30 ml) at room temperature for 2 hours. The solvent was removed and the resulting brown oil chromatographed on silica column. Elution with $CH_2Cl_2$ removed fast moving byproduct. Further elution with $CH_2Cl_2$:$CH_3OH$ (9.5:05), $R_f$ = 0.27, gave pure **2** as a light brown solid. Yield 132.8 mg (50%); mp 191-192 °C; $^{1}$H-NMR (400 MHz; $CDCl_3$) δ 9.77 (s, 1H), 8.08 (dd, 1H, J=8.9, 6.7 Hz), 7.70-7.39 (AB quartet, 4H), 6.98 (dd, 1H, J=11.6, 2.4 Hz), 6.57-6.52 (m, 1H), 2.80 (s, 3H); $^{13}$C-NMR (100 MHz; $CDCl_3$) ™ 170.76, 167.18 (d, $J_{C-F}$=253.3 Hz), 149.33 (d, $J_{C-F}$=12.2 Hz), 143.02, 139.50, 135.37 (d, $J_{C-F}$=12.2

Hz), 125.47, 122.32, 108.22, 106.35 (d, $J_{C-F}$=22.8 Hz), 100.69, (d, $J_{C-F}$=25.9 Hz), 43.75; MS (APCI+) 294 (M+1, 100); IR (KBr) 1673, 1592, 1228 $cm^{-1}$; Anal. calcd/found for: $C_{14}H_{12}FNO_3S$ C, 57.33/57.48; H, 4.12/4.27; N, 4.78/4.67.

EXAMPLE 3

4-Fluoro-2-(4-methanesulfonyl-phenylamino)-benzoic acid (**3**).

A solution of **1**(Scheme 1) (0.4458 g, 0.00152 mole) and tetrabutyl-ammonium oxon (1.1 g, 0.0030 mole) in $CH_2Cl_2$ (20 ml) was stirred at room temperature for 16 hours. TLC showed the presence of starting material; so additional 1.1 g (0.0030 mole) of the tetrabutylammonium oxon was added and reaction mixture stirred for 16 more hours. The reaction mixture was loaded on to a silica column and eluted with $CH_2Cl_2$:$CH_3OH$ (9.75:0.25) and the fast moving fraction collected and concentrated to give **3** as a white solid. Yield, 0.3856 g (82%); mp 200-202 °C; $^1$H-NMR (400 MHz; $CDCl_3$) δ 9.78 (s, 1H), 8.13 (dd, 1H, J=8.9, 6.5 Hz), 7.94-7.38 (AB quartet, 4H), 7.10 (dd, 1H, J=11.3, 2.4 Hz), 6.66-6.61 (m, 1H), 3.09 (s, 3H); $^{13}$C-NMR (100 MHz; $CDCl_3$) ™171.52, 167.28 (d, $J_{C-F}$=254.9 Hz), 148.32, 145.21, 135.59 (d, $J_{C-F}$=11.5 Hz), 134.50, 129.39, 120.62, 108.74, 107.46 (d, $J_{C-F}$=22.8 Hz), 101.61 (d, $J_{C-F}$=26.7 Hz), 44.78; $^{19}$F-NMR (376 MHz; $CDCl_3$) ™ -100.29 to -100.45 (m); MS (APCI+) 310 (M+1, 100); (APCI-) 308 (M-1, 100); Anal. calcd/found for: $C_{14}H_{12}FNO_4S$·0.75 $H_2O$ C, 52.08/52.36; H, 4.22/3.88; N,4.34/4.26.

EXAMPLE 4

2-methyl-4-trimethylsilanylethynyl-aniline (**5**)

To a solution of 4-iodo-2-methyl-aniline (2.33g, 10 mmol), bis(triphenylphosphine)palladium(II)chloride (1.4g, 0.2 mmol), CuI (0.19 g, 0.1 mmol) in $Et_3N$ (40 ml) at ice-bath temperature, (trimethylsilyl)acetylene (1.18 g, 12 mmol) was added dropwise (Scheme 2). After an hour stirring, the ice-bath was removed and the reaction mixture heated at 40°C (oil-bath temperature) for one hour; cooled to room temperature and the solvent removed. The residue was partitioned between $H_2O$ and $Et_2O$. The $Et_2O$ layer was separated, dried ($MgSO_4$) and concentrated to give an oil. The oil was purified by silica column,

eluting with $CH_2Cl_2$. The fraction with $R_f$ = 0.37 was collected and concentrated to give 2-methyl-4-trimethylsilanylethynyl-aniline as a dark brown oil. Yield 1.50 g (83%).

## EXAMPLE 5

4-Fluoro-2-(2-methyl-4-trimethylsilanylethynyl-phenylamino)-benzoic acid (**6**)

Continuing after Example 4, to a solution of 2-methyl-4-trimethyl-silanylethynyl aniline (1.50 g, 0.008 mole) in THF (10 ml) at ⁻78°C, LDA (2 M in THF, 6 ml, 0.012 mole) was added and the mixture was stirred at ⁻78°C for 30 minutes. Solid 2,4-difluoro-benzoic acid (0.633 g, 0.004 mole) was added and the stirred for 16 hours while it warmed up to room temperature. The solvents were removed and water (30 ml) and $Et_2O$ (50 ml) added to the oil residue. The mixture was stirred vigorously and the $Et_2O$ layer separated, dried ($MgSO_4$) and concentrated to give a brown solid. The solid was purified on silica column, eluted with $CH_2Cl_2$. The fraction with $R_f$ = 0.37 was collected and concentrated to give a light brown solid. The solid was added to pentane; some insoluble brown particulate was separated by filtration and discarded. The pentane layer was concentrated to give **6** as a light yellow solid. Yield 0.65 g (47%); mp 170-171°C; $^1$H-NMR (400 MHz; $CDCl_3$) δ 9.33 (s, 1H), 8.05 (dd, 1H, J=8.9, 6.8 Hz), 7.43 (d, 1H, J=1.2 Hz), 7.35 (dd, 1H, J=8.2, 1.7 Hz), 7.25 (d, 1H, J=8.2 Hz), 6.53 (dd, 1H, J=11.8, 2.4 Hz), 6.47-6.42 (m, 1H), 2.25 (s, 3H), 0.26 (s, 9H); $^{13}$C-NMR (100 MHz; $CDCl_3$) □ 172.86, 167.61 (d, $J_{C-F}$=253.3), 151.24 (d, $J_{C-F}$=12.3 Hz), 138.28, 135.38 (d, $J_{C-F}$=11.4 Hz), 134.85, 132.82, 130.63, 123.81, 119.91, 106.63, 105.23 (d, $J_{C-F}$=22.8 Hz), 104.77, 99.98 (d, $J_{C-F}$=26.7 Hz), 94.05, 17.78, 0.00; MS (APCI+) 342 (M+1, 100); IR (KBr) 2151, 1661, 1249 cm$^{-1}$; Anal. calcd/found for: $C_{19}H_{20}FNO_2Si$ C, 66.83/67.02; H, 5.90/6.00; N, 4.10/4.09; F, 5.56/5.45.

## EXAMPLE 6

4-Fluoro-2-(2-methyl-4-ethynyl-phenylamino)-benzoic acid (**7**).

To a solution of **6** in $CH_3OH$ (30 ml), aqueous 1N KOH (10 ml) was added. After stirring at room temperature for 16 hours, the $CH_3OH$ was removed and the

aqueous layer was acidified with 6N HCl (Scheme 2). The resulting white precipitation was extracted in to $Et_2O$, the $Et_2O$ layer was dried ($MgSO_4$) and concentrated to give **7** as tan colored solid. Yield 0.4274 g (91%); mp 177-178 ° C; $^1$H-NMR (400 MHz; $CDCl_3$) δ 9.35 (s, 1H), 8.08-8.04 (m, 1H), 7.44 (s, 1H), 7.38-7.25 (m, 2H), 6.57 (d, 1H, J=11.8 Hz), 6.48-6.44 (m, 1H), 3.08 (s, 1H), 2.27 (s, 3H); $^{13}$C-NMR (100 MHz; $CDCl_3$) ™ 172.84, 167.61 (d, $J_{C-F}$=253.3), 151.15 (d, $J_{C-F}$=12.3 Hz), 138.63, 135.40 (d, $J_{C-F}$=12.3 Hz), 135.00, 132.87, 130.81, 123.76, 118.79, 106.75, 105.33 (d, $J_{C-F}$=22.8 Hz), 100.03 (d, $J_{C-F}$=26.0 Hz), 83.37, 17.83, 0.00; $^{19}$F-NMR (376 MHz; $CDCl_3$) ™ -101.24 to -101.31 (m); MS (APCI+) 270 (M+1, 100); IR (KBr) 3315, 1672, 1594, 1253 $cm^{-1}$; Anal. calcd/found for: $C_{16}H_{12}FNO_2$ C, 71.37/71.08; H, 4.49/4.82; N, 5.20/5.09.

EXAMPLE 7

1-(4-nitro-phenyl)-1H-pyrrole (9a)

To a gently refluxing mixture of 4-nitroaniline (6.906 g, 0.05 mole), and sodium acetate (23 g, 0.28 mole) in acetic acid (100 ml) was added 2,5-dimethoxytetrahydrofuran (7.26 g, 7.12 ml, 0.055 mole) dropwise (Scheme 3). After refluxing for 3 hours, the reaction mixture was poured on to crushed ice (~250 ml), basified with 10 % sodium hydroxide (250 ml) and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was dried ($K_2CO_3$) to afford the product as a dark brown oil. Yield 9.40 g (100 %).

EXAMPLE 8

1-(4-nitro-phenyl)-1H-pyrazole (9b)

A mixture of pyrrazole (6.808 g, 0.1 mole) tetrabutylammonium bromide (3.22 g, 0.01 mole) and KOH (11.22 g, 0.2 mole) were ground together and sonicated for 16 hours. To this 1-fluoro-4-nitrobenzene (15.521 g, 11.67 ml, 0.11 mole) was added and the mixture sonicated for 24 hours. The reaction mixture was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was dried ($MgSO_4$) and concentrated to give dark brown solid. This was purified by silica column chromatography. Elution with $CH_2Cl_2$ ($R_f$ = 0.44) gave the product as a light

brown solid. Yield 8.80 g (47 %); mp 171-172 °C; Anal. calcd/found for: $C_9H_7N_3O_2$ C, 57.14/56.52; H, 3.73/3.62; N, 22.21/21.95.

EXAMPLE 9

3,5-dimethyl-1-(4-nitro-phenyl)-1H-pyrazole (9c)

To a solution of 4-nitro-phenyl-hydrazine (15.3 g, 0.1 mole) and 2,4-pentanedione (10.01 g, 10.27 ml, 0.1 mole) in EtOH (200 ml) were added 5 drops of concentrated HCl. The mixture was refluxed for 15 minutes; and the solvent removed to give a gummy product. This was purified by silica column chromatography. Elution with $CH_2Cl_2$ gave the desired product ($R_f$ = 0.10) as a brown solid. Yield 7.22 g (33 %).

EXAMPLE 10

4-Pyrrol-1-yl-phenylamine (10a)

Catalytic reduction ($H_2$/RaNi (5 g) /THF) of 1-(4-nitro-phenyl)- 1H-pyrrole (9.69 g, 0.05149 mole) at 51 psi gave crude product as an oil (Scheme 3). The product was purified by silica column chromatography. Elution with $CH_2Cl_2$ ($R_f$ = 0.13) gave the pure product as white solid. Yield 8.06 g (99 %); mp 80-81 °C.

EXAMPLE 11

In a manner similar to the preparation of 4-pyrrol-1-yl-phenylamine, the following were prepared:

4-1H-Pyrazol-1-yl-phenylamine (10b). Dark brown oil, yield 6.26 g (100 %).

Benzenamine, 4-(3,5-dimethyl-1H-pyrazzol-1-yl) (10c). Dark brown oil. Yield 6.45 g (100 %).

EXAMPLE 12

4-Fluoro-2-(4-pyrrol-1-yl-phenylamino)-benzoic acid (11a)

To a solution of 4-pyrrol-1-yl-phenylamine (3.16 g, 0.02 mole) in THF

(30 ml) at −78°C, a solution of LDA (2M, 15 ml, 0.03 mole) was added and the mixture stirred for 30 minutes. Solid 2,4-difluorobenzoic acid was added and the reaction mixture stirred for 16 hours as it warmed up to room temperature. The solvent was removed and ether (100 ml) added to the dark oily residue. This was stirred vigorously and the insoluble gummy precipitate separated by filtration. The gamy residue was dissolved in $H_2O$, acidified to pH 1 with 10% HCl, and extracted with $Et_2O$. The $Et_2O$ layer was dried ($MgSO_4$) and concentrated to give the target compound as a brown solid. Yield 2.74 g (93 %); mp 223-225 °C (DEC); $^{19}$F-NMR (376 MHz; $CDCl_3$) δ -101.44 (s); MS (APCI+) 297 (M+1, 100); IR (KBr) 1658, 1526, 1254 $cm^{-1}$.

In a manner similar to the preparation of 4-Fluoro-2-(4-pyrrol-1-yl-phenylamino)-benzoic acid, the following were prepared:

4-Fluoro-2-(4-pyrazol-1-yl-phenylamino)-benzoic acid (11b). Light brown solid, mp 212-213 °C.

2-[4-(3,5-Dimethyl-pyrazol-1-yl)-phenylamino]- 4-Fluoro benzoic acid (11c). Tan powder, mp 198 –200 °C.

## EXAMPLE 13

Cascade assay for inhibitors of the MAP kinase pathway

Incorporation of $^{32}$P into myelin basic protein (MBP) is assayed in the presence of a glutathione S-transferase fusion protein containing p44MAP kinase (GST-MAPK) and a glutathione S-transferase fusion protein containing p45MEK (GST-MEK). The assay solution contains 20 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM EGTA, 50,μM [γ-$^{32}$P]ATP, 10 μg GST-MEK, 0.5 μg GST-MAPK and 40 μg MBP in a final volume of 100 μL. Reactions are stopped after 20 minutes by addition of trichloroacetic acid and filtered through a GF/C filter mat. $^{32}$P retained on the filter mat is determined using a 120S Betaplate. Compounds are assessed at 10 μM for ability to inhibit incorporation of $^{32}$P.

To ascertain whether compounds are inhibiting GST-MEK or GST MAPK, two additional protocols are employed. In the first protocol, compounds are added to tubes containing GST-MEK, followed by addition of GST-MAPK, MBP and [$\gamma$-$^{32}$P]ATP. In the second protocol, compounds are added to tubes containing both GST-MEK and GST-MAPK, followed by MBP and [$\gamma$-$^{32}$P]ATP.

Compounds that show activity in both protocols are scored as MAPK inhibitors, while compounds showing activity in only the first protocol are scored as MEK inhibitors.

## EXAMPLE 14

In vitro MAP kinase assay

Inhibitory activity can be confirmed in direct assays. For MAP kinase, 1 $\mu$g GST-MAPK is incubated with 40 $\mu$g MBP for 15 minutes at 30°C in a final volume of 50 $\mu$L containing 50 mM Tris (pH 7.5), 10 $\mu$M $MgC1_2$, 2 $\mu$M EGTA, and 10 $\mu$M [$\gamma$-$^{32}$P]ATP. The reaction is stopped by addition of Laemmli SDS sample buffer and phosphorylated MBP resolved by electrophoresis on a 10% polyacrylamide gel. Radioactivity incorporated into MBP is determined by both autoradiography, and scintillation counting of excised bands.

## EXAMPLE 15

In vitro MEK assay

For evaluation of direct MEK activity, 10 $\mu$g GST-$MEK_1$ is incubated with 5 $\mu$g of a glutathione S-transferase fusion protein containing p44MAP kinase with a lysine to alanine mutation at position 71 (GST-MAPK-KA). This mutation eliminates kinase activity of MAPK, so only kinase activity attributed to the added MEK remains. Incubations are 15 minutes at 30°C in a final volume of 50 $\mu$L containing 50 mM Tris (pH 7.5), 10 $\mu$M $MgCl_2$, 2 , $\mu$M EGTA, and 10 $\mu$M [$\gamma$-$^{32}$P]ATP. The reaction is stopped by addition of Laemrnli SDS sample buffer. Phosphorylated GST-MAPK-KA is resolved by electrophoresis on a 10% polyacrylamide gel. Radioactivity incorporated into GST-MAPK-KA is determined by autoradiography, and subsequent scintillation counting of excised bands.

Additionally, an artificially activated MEK containing serine to glutamate mutations at positions 218 and 222 (GST-MEK-2E) is used. When these two sites are phosphorylated, MEK activity is increased. Phosphorylation of these sites can be mimicked by mutation of the serine residues to glutamate. For this assay, 5 μg GST-MEK-2E is incubated with 5 μg GST-MAPK-KA for 15 minutes at 30°C in the same reaction buffer as described above. Reactions are terminated and analyzed as above.

EXAMPLE 16

Whole cell MAP kinase assay

To determine if compounds block activation of MAP kinase in whole cells, the following protocol is used. Cells are plated in multi-well plates and grown to confluence. Cells are serum-deprived overnight. Cells are exposed to the desired concentrations of compound or vehicle (DMSO) for 30 minutes, followed by addition of a growth factor, for example, PDGF (100 ng/mL). After a 5-minute treatment with the growth factor, cells are washed with PBS, and lysed in a buffer consisting of 70 mM NaCl, 10 mM HEPES (pH 7.4), 50 mM glycerol phosphate, and 1% Triton X-100. Lysates are clarified by centrifugation at 13,000 x g for 10 minutes. Five to fifteen micrograms of protein from the resulting supernatants are subjected to SDS/PAGE and Western blotting for phosphorylated MAP kinase levels.

EXAMPLE 17

Monolayer growth

Cells are plated into multi-well plates at 10 to 20,000 cells/mL. Forty-eight hours after seeding, test compounds are added to the cell growth medium and incubation is continued for 2 additional days. Cells are then removed from the wells by incubation with trypsin and enumerated with a Coulter counter.

## EXAMPLE 18

Growth in soft-agar

Cells are seeded into 35-mm dishes at 5 to 10,000 cells/dish using growth medium containing 0.3% agar. After chilling to solidify the agar, cells are transferred to a 37°C incubator. After 7 to 10 days' growth, visible colonies are manually enumerated with the aid of a dissecting microscope.

## EXAMPLE 19

Collagen-Induced Arthritis in Mice

Type II collagen-induced arthritis (CIA) in mice is an experimental model of arthritis that has a number of pathologic, immunologic, and genetic features in common with rheumatoid arthritis. The disease is induced by immunization of DBA/1 mice with 100 µg type II collagen, which is a major component of joint cartilage, delivered intradermally in Freund's complete adjuvant. The disease susceptibility is regulated by the class II MHC gene locus, which is analogous to the association of rheumatoid arthritis with HLA-DR4.

A progressive and inflammatory arthritis develops in the majority of mice immunized, characterized by paw width increases of up to 100%. A test compound is administered to mice in a range of amounts, such as 20, 60, 100, and 200 mg/kg body weight/day. The duration of the test can be several weeks to a few months, such as 40, 60, or 80 days. A clinical scoring index is used to assess disease progression from erythema and edema (stage 1), joint distortion (stage 2), to joint ankylosis (stage 3). The disease is variable in that it can affect one or all paws in an animal, resulting in a total possible score of 12 for each mouse. Histopathology of an arthritic joint reveals synovitis, pannus formation, and cartilage and bone erosions. All mouse strains that are susceptible to CIA are high antibody responders to type II collagen, and there is a marked cellular response to CII.

EXAMPLE 20

SCW-induced monoarticular arthritis

Arthritis is induced as described by Schwab, *et al.*, *Infection and Immunity*, 59:4436-4442 (1991) with minor modifications. Rats receive 6 μg sonicated SCW [in 10 μl Dulbecco's PBS (DPBS)] by an intraarticular injection into the right tibiotalar joint on day 0. On day 21, the DTH is initiated with 100 μg of SCW (250 μl) administered i.v. For oral compound studies, compounds are suspended in vehicle (0.5% hydroxypropyl-methylcellulose/0.2% Tween 80), sonicated, and administered twice daily (10 ml/kg volume) beginning 1 hr prior to reactivation with SCW. Compounds are administered in amounts between 10 and 500 mg/kg body weight/day, such as 20, 30, 60, 100, 200, and 300 mg/kg/day. Edema measurements are obtained by determining the baseline volumes of the sensitized hindpaw before reactivation on day 21, and comparing them with volumes at subsequent time points such as day 22, 23, 24, and 25. Paw volume is determined by mercury plethysmography.

EXAMPLE 21

Mouse ear-heart transplant model

Fey, T.A. *et al.* describe methods for transplanting split-heart neonatal cardiac grafts into the ear pinna of mice and rats (*J. Pharm. and Toxic. Meth.* 39:9-17 (1998)). Compounds are dissolved in solutions containing combinations of absolute ethanol, 0.2% hydroxypropyl methylcellulose in water, propylene glycol, cremophor, and dextrose, or other solvent or suspending vehicle. Mice are dosed orally or intraperitoneally once, twice or three times daily from the day of transplant (day 0) through day 13 or until grafts have been rejected. Rats are dosed once, twice, or three times daily from day 0 through day 13. Each animal is anesthetized and an incision is made at the base of the recipient ear, cutting only the dorsal epidermis and dermis. The incision is spread open and down to the cartilage parallel to the head, and sufficiently wide to accommodate the appropriate tunneling for a rat or insertion tool for a mouse. A neonatal mouse or rat pup less than 60 hours old is anesthetized and cervically dislocated. The

heart is removed from the chest, rinsed with saline, bisected longitudinally with a scalpel, and rinsed with sterile saline. The donor heart fragment is placed into the preformed tunnel with the insertion tool and air or residual fluid is gently expressed from the tunnel with light pressure. No suturing, adhesive bonding, bandaging, or treatment with antibiotics is required.

Implants are examined at 10-20-fold magnification with a stereoscopic dissecting microscope without anesthesia. Recipients whose grafts are not visibly beating may be anesthetized and evaluated for the presence of electrical activity using Grass E-2 platinum subdermal pin microelectodes placed either in the pinna or directly into the graft and a tachograph. Implants can be examined 1-4 times a day for 10, 20, 30 or more days. The ability of a test compound to ameliorate symptoms of transplant rejection can be compared with a control compound such as cyclosporine, tacrolimus, or orally-administered lefluonomide.



## EXAMPLE 22

Murine ovalbumin-induced eosinophilia

Female C57BL/6 mice are obtained from the Jackson Laboratory (Bar Harbor, ME). All animals are given food and water ad libitum. Mice are sensitized with a single i.p. injection of OVA (grade V, Sigma Chemical Company, St. Louis, MO) adsorbed to alum, (10 μg OVA + 9 mg alum in 200 μl saline) or vehicle control, (9 mg alum in 200 μl saline) on day 0. On day 14, the mice are challenged with a 12-minute inhalation of an aerosol consisting of 1.5% OVA (weight/volume) in saline produced by a nebulizer (small particle generator, model SPAG-2; ICN Pharmaceuticals, Costa Mesa, CA). Groups of eight mice are dosed with oral vehicle (0.5% hydroxypropylmethylcellulose / 0.25% TWEEN-80), or a test compound at 10, 30, or 100 mg/kg in oral vehicle, 200 μl per mouse p.o. Dosing is performed once per day starting on day 7 or day 13, and extending through day 16.

For determination of pulmonary eosinophilia, three days after the first OVA aerosol challenge (day 17), the mice are anesthetized with an i.p. injection of anesthetic (Ketamine/Acepromazine/Xylazine), and the tracheae is exposed

and cannulated. The lungs and upper airways are lavaged twice with 0.5 ml of cold PBS. A portion (200 µl) of the bronchoalveolar lavage (BAL) fluid is enumerated using a Coulter counter Model ZB1 (Coulter Electronics, Hialeah, FL). The remaining BAL fluid is then centrifuged at 300 x g for five minutes, and the cells are resuspended in 1 ml of HBSS (Gibco BRL) containing 0.5% fetal calf serum (HyClone) and 10 mM HEPES (Gibco BRL). The cell suspension is centrifuged in a cytospin (Shandon Southern Instruments, Sewickley, PA) and stained by Diff Quick (American Scientific Products, McGraw Park, IL) to differentiate BAL leukocytes into neutrophil, eosinophil, monocyte or lymphocyte subsets. The number of eosinophils in the BAL fluid is determined by multiplying the percentage of eosinophils by the total cell count.

EXAMPLE 23

Caco-2 cell studies

Cell transport studies were conducted with Caco-2 cells grown on Snapwells between 22 to 28 days postseeding. Typically, 10 mM MES buffer (pH 6.5) with 5 mM KCl, 135 mM NaCl and 1.8 mM $CaCl_2$ was used for the apical side and 10 mM MOPS (pH 7.4) with 5 mM KCl, 132.5 mM NaCl and 1.8 mM $CaCl_2$ with 5 mM D-Glucose was used for the basolateral side. After washing the monolayers, appropriate buffers were pipetted into the respective chambers and the cells were pre-equilibrated at 37°C for at least 15 min. On the day of the experiment the growth media was aspirated and the cell monolayers were preequilibrated with appropriate buffers at 37°C for at least 15 min. Thereafter, TEER measurements were performed to confirm the integrity of the monolayers. Transepithelial flux measurements were made by mounting the cell monolayers in a side-by-side diffusion chamber system (Precision Instrument Design, Tahoe City, CA). Temperature was maintained at 37°C with a circulating water jacket. The solutions were mixed with gas-lift circulation with 95% oxygen-5% carbon dioxide. Donor solutions with PD compounds, [$^{14}$C] mannitol (leakage marker) and [$^{3}$H] metoprolol (reference compound) were added to the apical chamber. Donor and receiver samples were collected at selected time intervals for up to 3

hours. Radiolabelled mannitol and metoprolol were analyzed using scintillation counting (TopCount, Packard Instruments, Downers Grove, IL). PD compounds were analyzed using a LC/MS/MS method. Apparent permeability coefficients were calculated using the following equation:

$$P_{app} = (V^* dC) / (A.C_o. dt)$$

where V= volume of the receiver solution in ml, A = surface area in $cm^2$, $C_o$ = initial donor concentration in mM and dC/dt = change in the drug concentration in the receiver chamber over time.

EXAMPLE 24

Metabolic Stability in Human and Rat Liver Microsomes

Compounds are individually incubated (5 μM, dissolved in DMSO) with human and rat liver microsomes (0.5 mg/mL protein) in 50 mM KHPO4 buffer at 37°C in the presence of 1.0 mM NADPH. At 0, 10, 20 and 40 minutes, 100 μL aliquots are removed and added to 300 μL of acetonitrile. Standard curves are run in a similar manner with each compound at concentrations: 7.5 μM, 3.75 μM, 2.5 μM, 1.25 μM. The samples are analyzed for parent concentration by LC/MS/MS. The in vitro metabolic half-life determinations are determined from the concentration vs. time plots using WinNonlin. These in vitro data represent the rate of oxidative and hydrolytic metabolism.

EXAMPLE 25

Preparation of 2-(2-Chloro-4-iodo-phenylamino)-*N*-cyclopropylmethoxy-3,4-difluoro-benzenesulfonamide (PD 297447)

Step a: Preparation of *N*-Cyclopropylmethoxy-2,3,4-trifluoro-benzenesulfonamide

To a stirring suspension comprised of *O*-cyclopropylmethyl-hydroxylamine hydrochloride (5.40 g, 43.7 x $10^{-3}$ mol) in dichloromethane (20 ml) at ambient temperature under a nitrogen atmosphere was added directly diisopropylethylamine (10.8 ml, 0.062 mol). A solution comprised of 2,3,4-

trifluorobenzenesulfonyl chloride (1.00 g, 4.34 x $10^{-3}$ mol) in dichloromethane (120 ml) was added as a slow steady stream over twelve minutes to the first solution. After twelve minutes of stirring the combined reaction mixture, a 10 % aqueous hydrochloric acid solution (140 ml) was added. The biphasic mixture was stirred vigorously and the layers were separated. The organic phase was dried ($MgSO_4$) and concentrated to 6 ml volume. The concentrate was applied to a flash silica column (90 g of silica). Elution with dichloromethane afforded 0.83 g of the white amorphous solid product; 68 % yield; $^1$H-NMR (400 MHz; $CDCl_3$) δ 7.50 (m, 1H), 7.10 (s, 1H), 6.95 (m, 1H), 3.59 (d, 2H, J=7.2 Hz), 0.80 (m, 1H), 0.31 (m, 2H), 0.02 (m, 2H); $^{19}$F-NMR (376 MHz; $CDCl_3$) δ –122.65 (m, 1F), -129.37 (m, 1F), -156.20 (m, 1F); MS (APCI-) 280 (M-1, 100), 210 (55), 195 (45).

Step b: Preparation of 2-(2-Chloro-4-iodo-phenylamino)-*N*-cyclopropylmethoxy-3,4-difluoro-benzenesulfonamide

To a stirring solution comprised of 2-chloro-4-iodoaniline (0.80 g, 3.2 x $10^{-3}$ mol) in tetrahydrofuran (10 ml) at –78 °C under a nitrogen atmosphere was added 1.0 molar lithium *bis*trimethylsilylamide solution in tetrahydrofuran (6.2 ml, 6.2 x $10^{-3}$ mol) to form a green suspension. The suspension was stirred for five minutes before a stirring suspension comprised of lithiated *N*-cyclopropylmethoxy-2,3,4-trifluoro-benzenesulfonamide [prepared by adding 3.0 ml of the 1.0 molar lithium *bis*trimethylsilylamide solution to a stirring solution comprised of 0.83 g (2.95 x $10^{-3}$ mol) of *N*-cyclopropylmethoxy-2,3,4-trifluoro-benzenesulfonamide in 10 ml of tetrahydrofuran at –78 °C] was added. The cold bath was removed and the reaction mixture was stirred for one hour. Aqueous (10 %) hydrochloric acid (50 ml) was added to the reaction mixture, and the biphasic mixture was concentrated *in vacuo* to an aqueous suspension that was extracted with diethyl ether (200 ml). The ether phase was dried ($MgSO_4$) and concentrated *in vacuo* to afford 2 g of a tan oil. The crude product was purified by flash silica chromatography. Elution with a gradient [99:1 hexanes-ethyl acetate → (2 min) 9:1 → (25 min) 3:1] afforded 1.10 g of a clear amorphous foam; 73 % yield; $^1$H-NMR (400 MHz; DMSO) δ 7.69 (m, 1H), 7.59 (d, 1H, J=1.9

Hz), 7.34 (dd, 1H, J=8.7, 1.9 Hz), 7.27 (s, 1H), 7.00 (s, 1H), 6.95 (m, 1H), 6.43 (dd, 1H, J=8.7, 5.8 Hz), 3.52 (d, 2H, J=7.5 Hz), 0.74 (m, 1H), 0.34 (m, 2H), 0.02 (m, 2H); $^{19}$F-NMR (376 MHz; $CDCl_3$) $\delta$ –124.76 (m, 1F), -136.69 (d, 1F, J=18.3 Hz); MS (APCI+) 515 (M+1, 100); (APCI-) 513 (M-1, 50), 443 (73), 428 (100); IR (KBr) 1491 $cm^{-1}$; Anal. Calcd/found for $C_{16}H_{14}ClF_2IN_2O_3S$ C, 37.34/36.54; H, 2.74/2.71; N, 5.44/5.15; F, 7.38/7.57.

## F. OTHER EMBODIMENTS

From the above disclosure and examples, and from the claims below, the essential features of the invention are readily apparent. The scope of the invention also encompasses various modifications and adaptations within the knowledge of a person of ordinary skill. Examples include a disclosed compound modified by addition or removal of a protecting group, or an ester, pharmaceutical salt, hydrate, acid, or amide of a disclosed compound. Publications cited herein are hereby incorporated by reference in their entirety.

What is claimed is: